Microencapsulation of *Chromolaena odorata* Leaf Extract with Cellulose Esters for the Application as an Eco-Friendly Antibacterial Agent

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Abstract: *The aim of this work is to develop an eco-friendly antibacterial agent in the form of microcapsules containing extract from* Chromolaena odorata *leaves using the solvent evaporation method. The wall materials for encapsulating were tested with various cellulose esters including cellulose acetate (CA), cellulose acetate butyrate (CAB), and cellulose acetate propionate (CAP). The evaluation of microcapsules containing* C. odorata *leaf extract was focused on their encapsulation efficiency, size, shape, thermal stability, and antimicrobial activities. The results showed that CAB was a suitable wall material for the encapsulation of* C. odorata *leaf extract. The CAB microcapsules exhibited the highest encapsulation efficiency, which was 65.82 ± 3.07. The size of CAB microcapsules was the smallest, at 1013.3 ± 66.5 nm. According to the thermogravimetric analysis, the prepared microcapsules were able to protect the extract of* C. odorata *leaves from the environment. Moreover, the CAB microcapsules containing* C. odorata *leaf extract showed the best antibacterial activities against* Escherichia coli *ATCC 25922 and* Stapphylococcus aureus *ATCC 25923. The minimum bactericidal concentration of the microcapsules was 25.6 mg/mL in both bacteria. This study proved the potential application of* C. odorata *leaf extract as a biomaterial in diverse industries in the future.*

Keywords: Chromolaena odorata*; microencapsulation; antibacterial agent; cellulose ester*

■ INTRODUCTION

Consumers are now becoming more aware of the hygienic way of life, so the antimicrobial property is considered to be important. Commercial products are particularly likely to present natural or herbal products because they are eco-friendly [1]. Numerous antimicrobial materials, including synthetic and natural agents, have been developed to protect against microbial damage and prevent microbial infection [2]. Most products are made from synthetic agents, such as organo-metallic, phenols, quaternary ammonium salts, and organosilicons. Even though synthetic antimicrobial agents are highly effective at inhibiting microbes, they are hazardous to human health [3]. There are a lot of chemicals and heavy metals are non-biodegradable. Because of this, natural extracts are regarded as an alternative antibacterial agent for this work.

Chromolaena odorata (L.) King and Robinson (Asteraceae) is an invasive plant that is widespread throughout tropical Africa, North America, and South and Southeast Asia and is commonly known as Siam weed [4]. It is an herbaceous plant that forms a creeping bush of about 1.58–2.00 m [5-6]. The parts of *C. odorata* were used as a traditional medicine for colds, coughs, diarrhea, and inflammation of the skin and wounds [7-8]. The previous studies on the biological activities have shown that this plant has antidiabetic, anticataract [9], antifibrillogenic, hemolytic, cytotoxic, antioxidant [10-

11], anti-inflammatory [12], antinephropathic [13], and antifungal activities [14]. It also has larvicidal and repellent properties [15]. *C. odorata* has been investigated as an antimicrobial agent. It has been reported that the bioactive or phytochemical compounds of the extract from the leaf of *C. odorata* [16]. In addition, the antimicrobial activities of the extract of *C. odorata* leaves were effective against *Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes* and *Candida albicans* [17-20]. The antimicrobial activity of the leaf extract of *C. odorata* resulted from the secondary metabolites, which contained many important phytochemical compounds such as alkaloids, coumarins, flavonoids, phenols, saponins, and tannins [19-20]. However, the use of *C. odorata* leaf extract in industry was limited because the natural extract was unstable and oxidatively degraded [21]. To protect the antimicrobial activities from the bioactive compounds in the environment and for a long-lasting, the phytochemical compounds were applied in the microencapsulation technique.

Microencapsulation is the process of encapsulating the core inside the wall in the term of microcapsules, defined as particles with tiny spheres in the micrometer dimensions [22-23]. The encapsulation of the core, especially the active ingredients, protects them from the environment and controls their release through microencapsulation techniques. For this reason, microcapsules were used in various other areas, e.g., in the agricultural, construction, cosmetics, food, pharmaceutical, printing, and textile industries [24]. Currently, antimicrobial microcapsules are being developed that combine various natural extracts with polymers and are used as biopesticides, drugs, food preservatives, food packaging, textile finishing, and animal feed. For example, limonene and vanillin encapsulated by complex coacervation with a combination of chitosan and gum arabic [25], Chinese nutgall encapsulated with sodium alginate and chitosan by orifice granulation [26], carvacrol encapsulated with pectin and alginate by spray drying [27], thyme oil encapsulated with gum arabic [28], citrus oil encapsulated with chitosan and modified starch by spray drying [29], lime oil encapsulated with alginate and gelatin by coacervation [30], cinnamon oil encapsulated with a xanthan gum/chitosan composite [31], peppermint oil with a gelatin-based cryogel [32], and propolis encapsulated with gum arabic and β-cyclodextrin by spray drying [33].

In this work, the wall material of interest was cellulose esters, the cellulose derivatives of which were one of the most commonly used wall materials for microcapsules. Due to the excellent properties of cellulose esters, including low toxicity, high stability, high T_g, and film strength, they could form micro-and nanoparticles [34]. Cellulose esters are also nature-friendly materials, so they are obtained from the esterification of renewable and biodegradable cellulose from biomass [35]. Various types of organic cellulose esters have been used in previous research, such as cellulose acetate (CA), cellulose acetate propionate (CAP), cellulose acetate butyrate (CAB), and cellulose acetate trimellitate. In this study, CA, CAB and CAP were used as wall materials to encapsulate *C. odorata* leaf extract due to their semi-permeable property. These cellulose esters are insoluble in water and can be used as hydrophobic films for encapsulation. They can create a semi-permeable wall membrane to control the release of microcapsules [36]. In this research, the type of cellulose esters was varied because the type of ester cellulose groups affects the physical properties of the synthesized microcapsules [37].

The aim of this work was to investigate a suitable cellulose ester to encapsulate the *C. odorata* leaf extract using a solvent evaporation method to develop an eco-friendly antimicrobial agent. The synthesized microcapsules were analyzed for their percentage yield and encapsulation efficiency. The morphology was characterized by scanning electron microscopy (SEM). The mean value of the particle size was estimated with a particle size analyzer (PSA). The thermal properties were investigated using the thermogravimetric analyzer (TGA). Finally, the efficacy of antibacterial activities against *E. coli* and *S. aureus* was evaluated by using broth dilution and spreading plates.

■ EXPERIMENTAL SECTION

Materials

CA (number molecular weight, Mn~30,000, wt.% acetyl = 39.8, d =1.3 g/mL), CAB (Mn~30,000, 12.0–15.0 wt.% acetyl, 36.0–40.0 wt.% butyryl, d = 1.25 g/mL), CAP (Mn~75,000, 46 wt.% propionyl, d = 1.23 g/mL), and dimethyl sulphoxide (DMSO) were supplied by Sigma-Aldrich. Poly(vinyl alcohol) (PVA) was purchased by Ajex Finchem. Chloroform, ethanol, and methanol were obtained from Labscan. The nutrient broth medium was from Himedia. Cellulose esters were R&D grade, and other chemicals were laboratory reagents.

Instrumentation

The preparation of microcapsules was performed by using the IKA RW20 Overhead stirrer, respectively. The encapsulation efficiency (%EE) of *C. odorata* was assessed by using a GBC Scientific Equipment UV-vis spectrophotometer. The characterization of microcapsules was analyzed by using TESCAN VEGA3 SEM, Haribo SZ-100 PSA, and Thermoplus TG 8120, Rigaku TGA.

Procedure

C. odorata *leaf extract preparation*

The fresh leaves of *C. odorata* were washed with water to remove soil residues and dust particles. They were then dried in full sunlight for 1–2 d. The 500 g of dried leaves were subsequently fragmented and subjected to extraction using 5 L of 95% ethanol for 24 h, with a weight-to-volume ratio of leaves to ethanol set at 1:10. Once the extract was thoroughly mixed using an electric blender, it was then passed through a filter cloth and subsequently through Whatman filter paper No. 1. Later, the filtrate was concentrated by evaporation of ethanol and freeze-dried. Finally, the crude extract obtained from the leaves was in the form of green powder and stored in a refrigerator at 4 °C for further studies.

Microencapsulation of C. odorata *leaf extract*

Microcapsules containing *C. odorata* leaf extract were prepared by oil-in-water emulsion solvent evaporation according to the method of Simões et al. [38] with some modifications. First, the organic phase was prepared by dissolving 1.2 g of *C. odorata* leaf extract and 1.2 g of cellulose ester (1:1) in 40 mL of a 50/50 (v/v) mixture of chloroform/ethanol. After adding the organic phase to the aqueous phase, which consisted of 200 mL of a 0.5% w/v PVA solution, the mixture was homogenized for 6 min at 12,000 rpm using a high-speed homogenizer. The resulting oil in water emulsions was then continuously stirred at 1,300 rpm with an overhead stirrer for 6 h at room temperature to allow the organic solvent to evaporate. Afterward, the obtained microcapsules were collected by centrifugation at 10,000 rpm for 30 min and washed 3 times with deionized water. Finally, the prepared microcapsules containing *C. odorata* leaf extract were dried at room temperature in a desiccator and stored under vacuum conditions.

In this process, the *C. odorata* leaf extract was combined as the core material and the cellulose ester as the wall material by dissolving in an organic solvent. The homogenous solution was then added to the PVA solution as an emulsifier to create an oil-in-water emulsion and mixed using high-speed homogenization, which allowed the emulsion to be broken into a smaller size. Furthermore, the resulting emulsion was continuously stirred to evaporate the organic solvent. In the meantime, the evaporation of the solvent led to the formation of a wall on the droplets of the microcapsules. Finally, the insoluble microcapsules were separated from the water and agglomerated at the bottom [39-40].

Determination of encapsulation yield and encapsulation efficiency

First, the encapsulation yield (EY, %) of the synthesized microcapsules was calculated from the percentage of the weight of the synthesized microcapsules and the total added materials, including the *C. odorata* leaf extract, PVA, and cellulose ester, as shown in Eq. (1).

$$\%EY = \frac{\text{Weight of synthesized microcapsules}}{\text{Weight ot total added materials}} \times 100 \quad (1)$$

Secondly, the determination of *C. odorata* leaf extract content in the microcapsules was carried out as follows: 0.05 g of the prepared microcapsules containing *C. odorata* leaf extract was dissolved in 10 mL of ethanol/methanol (1:1 v/v) solvent mixture. The microcapsule solution was sonicated at 20 kHz and

200 W energy input for 30 min with ultra-sonication and centrifuged at 10,000 rpm for 30 min, respectively. The supernatant was collected and analyzed with a UV spectrophotometer at 650 nm. The content of encapsulated *C. odorata* leaf extract was determined from the concentration curve of *C. odorata* leaf extract and used to determine the %EE according to Eq. (2). The %EE is defined as the ratio between the experimentally determined *C. odorata* leaf extract content and the theoretical *C. odorata* leaf extract loading.

$$\%EE = \frac{\text{Weight of extract in microcapsules}}{\text{Weight of added extract}} \times 100 \quad (2)$$

Microcapsule characterization

First, the morphology of the microcapsules was examined with the SEM. The microcapsule samples were fixed on a double-sided black adhesive tape next to an SEM stub. Before the analysis, the surface of the microcapsule was coated with a thin layer of gold under vacuum.

Secondly, the mean size and particle size distribution of the microcapsules were determined using a PSA based on the dynamic light scattering technique. The microcapsules were suspended in distilled water at 25 °C, as this does not interact with the particles. The test was repeated 10 times per sample.

Finally, the thermal stability of the extracts, the microcapsules without extract, and the microcapsules containing extract were analyzed using the thermogravimetric analyzer. The samples were analyzed at a heating rate of 10 °C/min from 27–600 °C under nitrogen conditions. The thermograms showed the weight loss during composition at different temperatures.

Antibacterial assay

The antibacterial activity of the microcapsules was investigated by determining the minimum inhibitory concentration (MIC) using the broth dilution method and the minimum bactericidal concentration (MBC) using spreading plates. The test was performed with two types of bacteria, including the Gram-negative bacterium *E. coli* ATCC 25922 and the Gram-positive bacterium *S. aureus* ATCC 25923. The antibacterial activity of the microcapsules against *E. coli* and *S. aureus* was assessed by determining the MIC using the broth dilution method and the MBC using a spreading plates technique. The experimental procedure recommended by Amini Tapouk et al. [41] was followed with some modifications. Microcapsule solution was diluted in a series of concentrations ranging from 51.2, 25.6, 12.8, 6.4, 3.2, 1.6, 0.8, 0.4, 0.2, and 0.1 mg/mL using DMSO as the diluent. The dilution was done in a two-fold manner. To prepare the bacterium for testing, a single colony of the bacterial culture was transferred into a nutrient broth and incubated at a temperature of 37 °C for a period of 18–24 h. Next, the density of the bacterial suspensions was adjusted to the 0.5 McFarland Standard, which is approximately 1.5×10^8 CFU/mL. Following that, the suspensions were diluted with a 0.85% w/v sterile saline solution to obtain a concentration of 1.5×10^5 CFU/mL. Afterwards, 0.5 mL of the freshly prepared bacterial suspensions of the test organisms was added into 1.0 mL of the microcapsule solutions. The test sample comprised 2 negative controls consisting of a solution of *C. odorata* leaf extract with a concentration of 51.2 mg/mL and a bacterial suspension and a solution of microcapsules with a concentration of 51.2 mg/mL and a bacterial suspension. Meanwhile, the positive control consisted of bacterial suspension and nutrient broth. Following an incubation period of 24 h of the bacterial strains at a temperature of 37 °C, the absence of turbidity or the presence of clear solutions were observed and measured as MIC in comparison to the negative and positive controls. The MIC was determined to be the lowest concentration of the microcapsules and could effectively inhibit the growth of each bacterium. Subsequently, the MBC determination was carried out by spreading the clear solutions of MIC onto a nutrient agar plate. After incubating the cultured plates at 37 °C for 24 h, the presence of colonies was observed to determine the MBC. The MBC was determined as the lowest concentration at which the bacteria were completely killed.

■ RESULTS AND DISCUSSION

Microcapsule Preparation

Different cellulose esters (CA, CAB, and CAP) were used to prepare microcapsules containing *C.*

odorata leaf extract using an oil-in-water process. The prepared microcapsules were determined %EY and %EE. The weight of the synthesized microcapsules with CA, showed a remarkably significant difference from the microcapsules synthesized with CAB and CAP, as presented in Table 1. The %EY of CAB and CAP microcapsules was higher than that of CA microcapsules. As for the encapsulation efficiency, the CAB microcapsules exhibited the highest %EE, while the CA microcapsules could contain the lowest of *C. odorata* leaf extract. These results indicated that CAB and CAP were more suitable as wall material than CA. This might depend on the nature of the substituents. The chemical structures of the three cellulose esters, as shown in Fig. 1, consist of a cellulose backbone and substituted groups attached to the hydroxyl groups. The substituted groups of CA, CAB, and CAP were acetyl groups, a combination of acetyl and butyrate groups, and a combination of acetyl and propionate groups, respectively [42-44]. The size of the acyl groups could affect on the packing density and polarity of the cellulose chains [45]. The higher the number and size of the substituted groups, the lower the polarity of the cellulose esters. Thus, CAB could be dissolved in organic solvents such as chloroform and ethanol, which were better represented as CAP and CA, respectively, in this work. In addition, the extract from the leaves of *C. odorata* was highly soluble in organic solvents and fused strongly with CAB to remain in the microcapsules after evaporation of the solvent. Consequently, the microcapsules prepared with CAB and CAP yield higher %EE and %EY than the CA one. The values of %EY and %EE serve as indicators of the performance and quality of microcapsule encapsulation. Furthermore, the wall materials with higher %EY were able to effectively encapsulate the extract, resulting in the production of microcapsules. Alternatively, the initial materials could be used to produce high microcapsules. A higher %EE indicates successful encapsulation of the extract during the process. Therefore, the outcomes of elevated %EY and %EE were more desirable in the experiments [46].

Microcapsule Characterization

The prepared microcapsules were primarily analyzed regarding their morphology and size distribution. The size distribution curves show considerable differences depending on the cellulose esters used. As the results in Fig. 2 show, the CA, CAB, and CAP microcapsules are spherical particles corresponding to the work of Wondraczek et al. [47]. However, the surface of the resulting microparticles differed slightly. Fig. 2(a) illustrates a smooth surface with a small pore of the microcapsules encapsulated with CA. The morphology of the CA microcapsules was consistent with the results of Guastaferro et al. [48]. They investigated that the CA concentration of 1 %w/w helped to improve the sphericity of the nanoparticles.

The microcapsules encapsulated with CAB exhibited a coarser and rougher surface (Fig. 2(b)). In addition, the microcapsules prepared with CAP had a significantly porous and holey surface (Fig. 2(c)). They indicate that the type of cellulose esters could influence the coating of the extract, as they form a wall material on the outside. Previous studies have reported that a thin layer of cellulose ester forms when the solvent evaporates. The microcapsules became solid water-insoluble particles after the solvents were removed from oil-in-water emulsion [49]. The hydrophobic acetyl groups of the cellulose esters were favored toward the core of the microcapsules, while the hydrophilic hydroxyl groups of the cellulose esters were directed outward.

Table 1. Encapsulation yield and encapsulation efficiency of microcapsules containing *C. odorata* leaf extract prepared with three cellulose esters

Type of cellulose ester	%EY	%EE
CA	44.97 ± 2.32	51.11 ± 1.90
CAB	46.79 ± 2.97	65.82 ± 3.07
CAP	47.79 ± 2.27	63.90 ± 3.27

The experiments were performed in triplicate

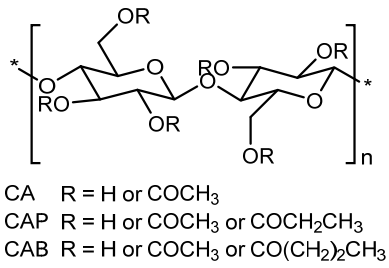

Fig 1. The chemical structure of cellulose ester derivatives

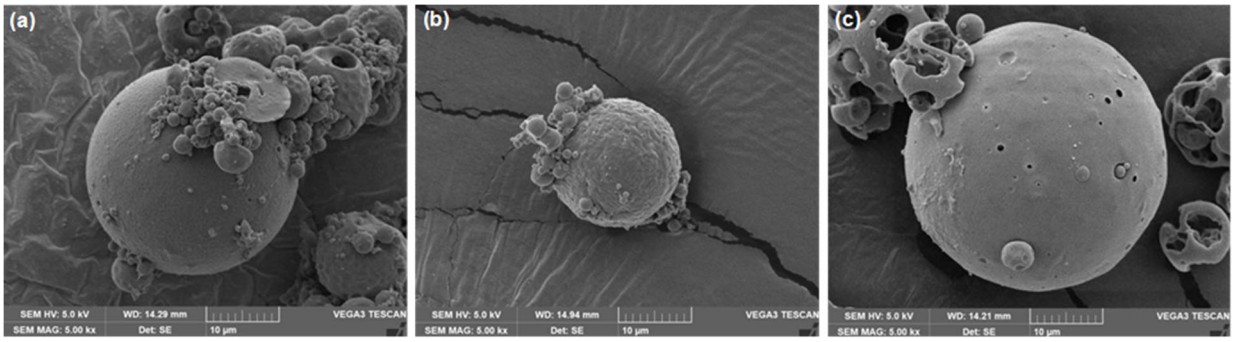

Fig 2. SEM images of microcapsules containing *C. odorata* leaf extract encapsulated with three cellulose esters (a) CA, (b) CAB, and (c) CAP, respectively

The precipitation of microcapsules that dissolved in the organic solvents was driven by water and took place at the interface between water-organic solvents [50]. The permeation property of cellulose esters was caused by the interaction between cellulose esters and water molecules [51]. Meantime, the solvents diffused outward through the thin layer of cellulose ester, while the water diffused into the microcapsules [52]. Therefore, the porous wall was created in this process and appeared on the surface layer. The hydrophobicity of CAB and CAP increased according to the type of acetyl size. The CAB microcapsules comprised the butyrate group, which was the largest acetyl group, so they had the strongest interaction with water molecules [53]. This resulted in CAB microcapsules having a coarser surface area than CA and CAP microcapsules. The porosity of CAB microcapsules observed in this experiment was consistent with the work of Baldelli et al. [39]. This showed that the physical properties of the microcapsules were created during the particle production.

The results of the size distribution of the microcapsules were described by plotting the particle diameter (nm) against the percentage of frequency. Fig. 3 shows that the particle diameter of the microcapsules containing *C. odorata* leaf extract encapsulated with CA, CAB and CAP were in the range of 1050–1600, 800–1400, and 3200–4200 nm, respectively. The mean diameters of microcapsules encapsulated with CA, CAB, and CAP were 1191.6 ± 82.0, 1013.3 ± 66.5, and 3422.7 ± 81.7 nm, respectively. The results showed that the microcapsules encapsulated with CA and CAB have a smaller particle size and a relatively narrow size distribution than the

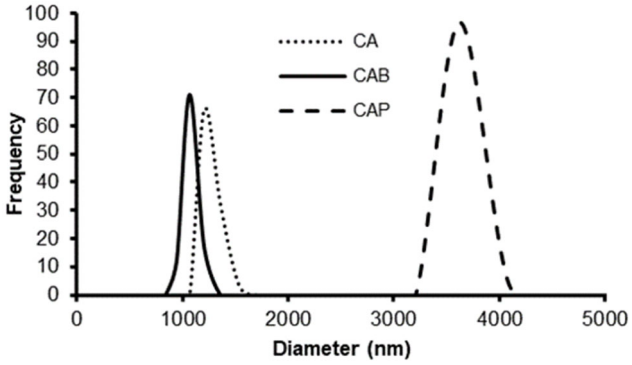

Fig 3. Particle size distribution of microcapsules containing *C. odorata* leaf extract encapsulated with three cellulose esters

microcapsules prepared with CAP. These results agree with the experiments of Onda et al. [54], who reported that the particle size of the microcapsules increased with increasing polymer molecular weight. The higher molecular weight of the polymer influenced the viscosity of the internal phase of the polymer solution, which was higher. As the polymer had a resistance to break down into smaller fragments, this led to the formation of larger microcapsules. The number molecular weight of CA and CAB studied in this work was about 30000, while CAP was about 75000. Therefore, smaller particles were formed from the CA and CAB microcapsules than the CAP microcapsules. Besides, the CAB microcapsules were slightly smaller than the CA microcapsules, which may be due to the internal viscosity of the polymer. When the internal viscosity of the polymer increased, this led to slow agglomeration in the microcapsules, increasing the particle size of microcapsules [55]. From the product data sheet, the viscosity value of CA

(1.3 g/mL) at 25 °C was higher than that of CAB (1.25 g/mL), so that CAB could form a smaller microcapsule size. These results agreed with the report of Simões et al. [38] that microspheres with CA and CAB formed smaller particles than microspheres with CAP.

Fig. 4(a) illustrates a TGA diagram of the extract from the leaves of *C. odorata*, which begins to decompose at 70 °C due to water evaporation. The subsequent decomposition of the *C. odorata* leaf extract obviously occurred at 120 °C, and the weight loss was continuously up to 400 °C. This result suggests that the decomposition of *C. odorata* leaf extract started at 120 °C. For the thermal characterization of the microcapsules containing *C. odorata* leaf extract encapsulated with three cellulose esters, they were compared with the microcapsules without the extract and described in Fig. 4(b–d) and Table 2. The TGA diagrams of the microcapsules containing *C. odorata* leaf extract encapsulated with three cellulose esters showed similar thermal behavior to the TGA diagrams of the microcapsules without the extract. The TGA diagrams of the whole microcapsules were observed in 3 stages: the first stage was at 30–120 °C, the next stage was at 250–400 °C, and the third stage was at 450–550 °C. A slight weight loss at 70–120 °C in the first stage was due to water evaporation, as suggested by the TGA diagram of the *C. odorata* leaf extract. In the second stage, the TGA diagrams of the microcapsules without the extract prepared with CA, CAB, and CAP in Fig. 4(b–d) show an extrapolated onset temperature at 338.1, 347.3, and 330.5 °C, respectively, and a maximum decomposition at 373.8, 380.3, and 372.9 °C, respectively. These decompositions of the microcapsules were caused by the degradation of acyl, butyryl, and propionyl groups associated with the cellulose backbone, by the degradation of the cellulose main chain [55-57] and by the evaporation of bound water [58] via thermo-oxidative degradation [57] and continued until the maximum decomposition temperature. The microcapsules containing the extract encapsulated with CA, CAB, and CAP showed an extrapolated onset temperature of 329.7, 336.7, and 315.2 °C, respectively, and a maximum decomposition of 376.9, 385.3, and 376.9, respectively.

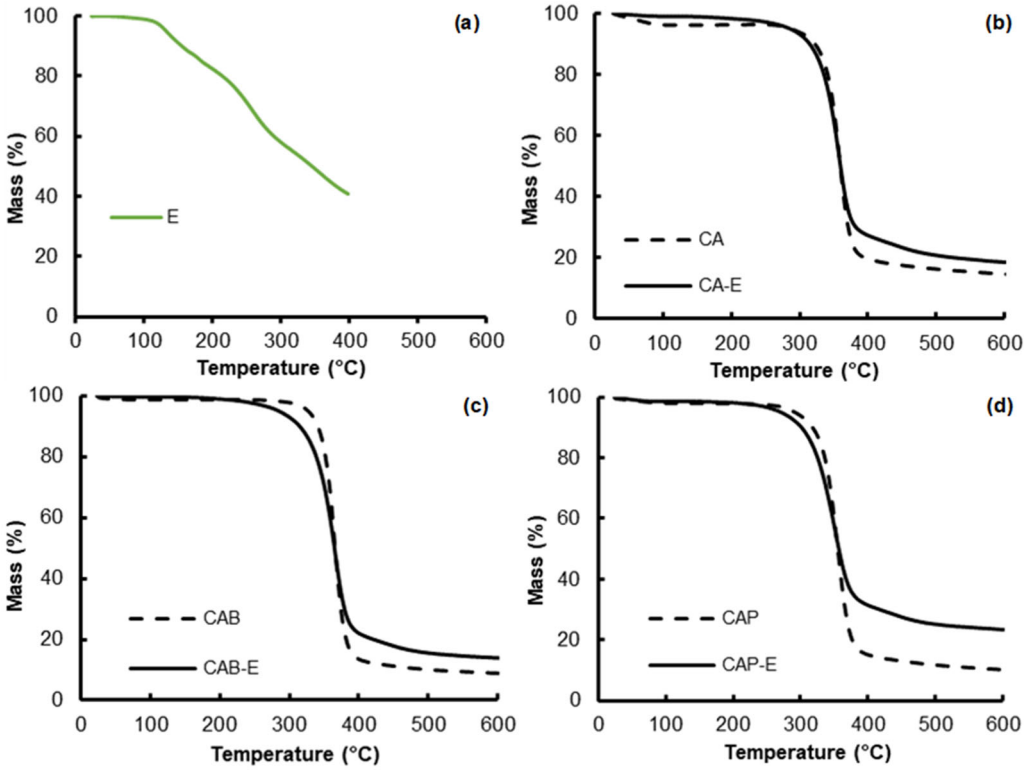

Fig 4. TGA curves of (a) *C. odorata* leaf extract, (b) microcapsules without/containing *C. odorata* leaf extract encapsulated with CA, (c) CAB, and (d) CAP, respectively

Table 2. TGA data of microcapsules without and containing *C. odorata* leaf extract encapsulated with three cellulose esters from the TGA diagrams

Type of cellulose ester	Onset decomposition temperature (°C)	Maximum decomposition temperature (°C)	Weight loss (%)
CA	338.10	373.80	82.34
CA-E	329.70	376.90	80.86
CAB	347.30	380.30	89.86
CAB-E	336.70	385.30	85.90
CAP	330.50	372.90	87.79
CAP-E	315.20	376.90	75.04

It could be seen that the onset temperature of decomposition was lower for the microcapsules without the extract, and the maximum decomposition temperature was higher for the microcapsules containing the extract. These results indicated that the extract of *C. odorata* leaves contained in the microcapsules was also decomposed simultaneously. The results of this experiment were consistent with the studies of Alghamdi and El-Zahhar [58], which found the degradation of cellulose acetate butyrate-graphene oxide nanocomposite. Watanabe et al. [59] pointed out that the initial decomposition temperature of CA microcapsules loaded with *n*-hexadecane was lower than pure CA. Furtado et al. [60] also reported that the maximum decomposition temperature of CAB-caffeine film was higher than that of pure CAB. The weight loss of the microcapsules from the temperature of the onset of decomposition to the maximum decomposition temperature at this stage could be determined, as shown in Table 2. Finally, the decomposition of the char residue in the third stage due to the pyrolysis was observed around 450–550 °C [60]. It could be seen that microcapsules containing *C. odorata* leaf extract began to decompose at a temperature of 250 °C, which was higher than that of the *C. odorata* leaf extract that decomposed at 120 °C. These results suggested that cellulose esters could protect the extract from heat so that the microcapsules had better resistance to heat damage. The order of thermal stability of the three cellulose esters used as wall material for encapsulation *C. odorata* leaf extract was CAB, CA, and CAP, respectively, which is consistent with the result of Simões et al. [38]. Comparing the temperature of weight loss of the microcapsules containing *C. odorata* leaf extract encapsulated with CA, CAB and CAP, the weight loss of the CAB microcapsules was higher than CA or CAP microcapsules. This could be due to the porous surface of the CAB microcapsules, which caused the extract to evaporate more easily than in the CA microcapsules, as seen in Fig. 2.

Antibacterial Activity

Evaluation of the antibacterial activity of microcapsules containing *C. odorata* leaf extract encapsulated with three cellulose derivatives, CA, CAB, and CAP. The experiments were performed by broth dilution method with *E. coli* and *S. aureus* were used as model bacteria. The MBC of microcapsules containing *C. odorata* leaf extract prepared with CA, CAB, and CAP were 51.2, 25.6, and 51.2 mg/mL against *E. coli*, respectively, as shown in Fig. 5(a–c) and Table 3. CAB microcapsules containing *C. odorata* leaf extract were more effective against *E. coli* than microcapsules containing chambá extract encapsulated with maltodextrin [61] and microcapsules containing citrus oil encapsulated with a modified starch-chitosan matrix [28], which had MBC of 200 and 28 mg/mL, respectively. However, microcapsules containing propolis extract encapsulated with β-cyclodextrin and gum Arabic [33]

Table 3. MBC of microcapsules containing *C. odorata* leaf extract encapsulated with three cellulose esters

Type of cellulose ester	MBC (mg/mL)	
	E. coli	*S. aureus*
CA	51.2	51.2
CAB	25.6	25.6
CAP	51.2	51.2

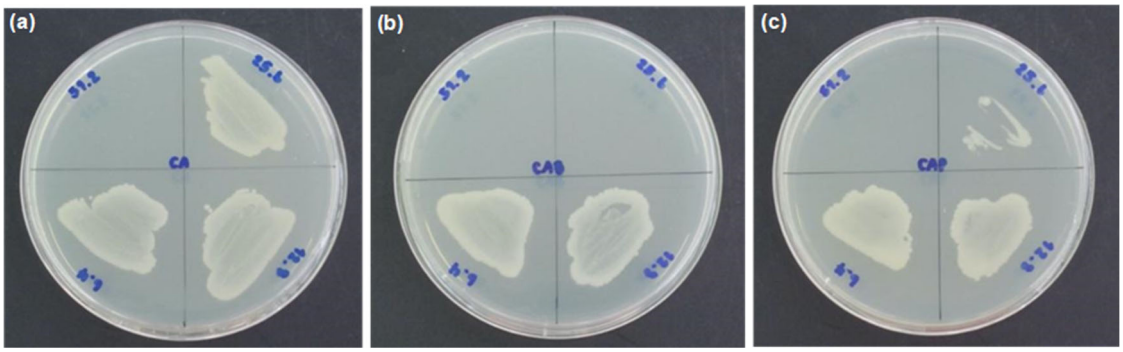

Fig 5. Antibacterial activity against *E. coli* of microcapsules containing *C. odorata* leaf extract encapsulated with cellulose esters (a) CA, (b) CAB, and (c) CAP, respectively

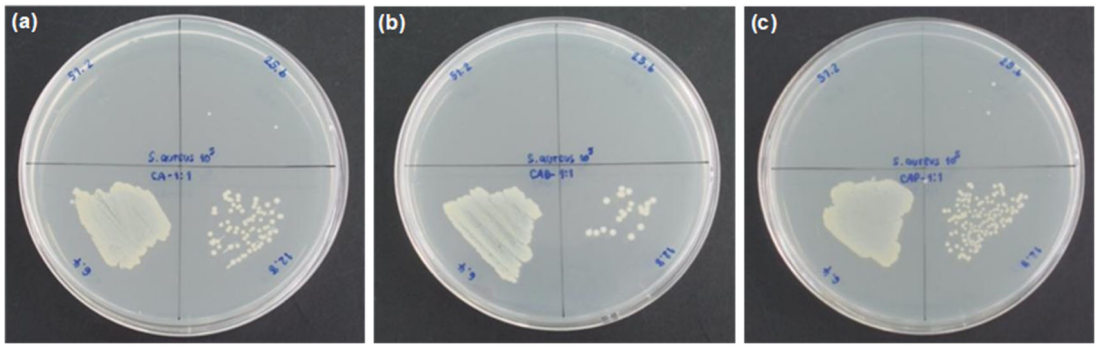

Fig 6. Antibacterial activity against *S. aureus* of microcapsules containing *C. odorata* leaf extract encapsulated with three cellulose esters (a) CA, (b) CAB, and (c) CAP, respectively

and microcapsules containing carvacrol encapsulated with a pectin-alginate matrix [27] showed better antibacterial activity than CAB microcapsules containing *C. odorata* leaf extract and their MBC and MIC were 1.25, and 0.25 mg/mL, respectively.

The results of antibacterial activity against *S. aureus*, showed that microcapsules containing *C. odorata* leaf extract encapsulated with CAB killed bacteria growth at 25.6 mg/mL more than microcapsules containing *C. odorata* leaf extract encapsulated with CA and CAP (51.2 mg/mL) as shown in Fig. 6(a–c) and Table 3. Nevertheless, the antibacterial activity of CAB microcapsules containing *C. odorata* leaf extract was weaker against *S. aureus* than that of microcapsules containing chambá extract encapsulated with maltodextrin [61] and microcapsules containing propolis extract encapsulated with β-cyclodextrin [29] which were able to kill *S. aureus* at 5, and 0.30 mg/mL, respectively.

The results showed that the CAB microcapsules had the highest antibacterial effect against *E. coli* and *S. aureus*. Therefore, the CAB microcapsules were more suitable for further use. The antibacterial effect was related to the phytochemicals in the leaf extract of *C. odorata* [62]. These phytochemicals included phenolic compounds, flavonoids, tannins, alkaloids, saponins, terpenes, and various trace substances [63]. Lobiuc et al. [64] described the antibacterial mechanism of action of phenolic compounds that consist of a hydroxyl functional group (–OH) which represents a negative charge. It was hypothesized that the increase in hydroxyl groups on the phenyl group was related to their relative toxicity to bacteria. The hydroxylation destroyed the cytoplasmic membrane and led to cell death. Flavonoids could bind to the bacterial cell wall, which in effect inhibits the synthesis of the cell wall and effectively hinders bacterial growth [20]. Tannins possess the capacity to induce the precipitation of proteins, thereby impeding the ability of microorganisms to adhere. Moreover, they were inhibiting the activity of enzymes and proteins that are responsible for the transportation

of substances across the cellular membrane. Alkaloids were found to interfere with cell division and bind to DNA through intercalation [18]. In addition, it was speculated that the mechanism of terpenes, which belonged to lipophilic compounds, was related to membrane disruption and permeability [65]. Although, the MBC value against *E. coli* and *S. aureus* of the CAB microcapsules containing *C. odorata* leaf extract was in the same range. However, Gram-positive bacteria (*S. aureus*) were more sensitive than Gram-negative bacteria (*E. coli*), as indicated by the growth intensity of the microorganisms (see Fig. 5(b) and Fig. 6(b)). Previous studies have shown that phenolic compounds were more effective in Gram-positive bacteria than in Gram-negative bacteria due to their characteristic cell wall. Gram-positive bacteria have a thinner peptidoglycan outer layer of the cell wall. In contrast, the outer layer of the cell wall of Gram-negative bacteria is a phospholipid membrane, which consists of lipopolysaccharides and is impermeable to hydrophobic substances [61,66]. In addition, the cell membrane of Gram-positive bacteria is more porosity, allowing some bioactive compounds to spread inside and attack the cell membrane [67-68].

■ CONCLUSION

The solvent evaporation method has been employed to successfully construct an eco-friendly antibacterial agent by encapsulating *C. odorata* leaf extract with three cellulose esters: CA, CAB, and CAP. The use of CAB as a wall material for encapsulating *C. odorata* leaf extract demonstrated superior qualities compared to CA and CAP. This was evident in terms of its higher encapsulation efficiency, enhanced thermal stability, and stronger antibacterial properties. Furthermore, the particle size distribution of microcapsules containing *C. odorata* leaf extract, prepared using CAB, was smaller compared to microcapsules prepared using CA and CAP. This smaller particle size distribution is advantageous for future applications. Nevertheless, the CAB microcapsules containing *C. odorata* leaf extract were deemed worthy of further development to enhance their antibacterial efficacy. This research attended the advantage of utilizing an invasive plant found in Thailand, which is widespread globally, as a biomaterial with potential applications in various industries in the future.

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■ CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

■ AUTHOR CONTRIBUTIONS

Jiraporn Ketwaraporn: conceptualization, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, validation, visualization, writing–original draft, writing–review & editing. Somyod Pinthong, Rungnapha Kerdphu, and Surahani Daebau: microcapsule preparation experiments and analysis. Parinya Kraivuttinun: antibacterial activity experiments and analysis, contributed reagents and materials, writing–review & editing. Pongthep Jansanthea: methodology, validation, writing–review & editing.

■ REFERENCES

[1] Das, A., and Satyaprakashj, K., 2018, Antimicrobial properties of natural products: A review, *Pharma. Innovation*, 7 (6), 532–537.

[2] Koh, E., and Hong, K.H., 2014, Gallnut extract-treated wool and cotton for developing green functional textiles, *Dyes Pigm.*, 103, 222–227.

[3] Karypidis, M., Karanikas, E., Papadaki, A., and Andriotis, E.G., 2023, A mini-review of synthetic organic and nanoparticle antimicrobial agents for coatings in textile applications, *Coatings*, 13 (4), 693.

[4] Anyanwu, S., Inyang, I.J., Asemota, E.A., Obioma, O.O., Okpokam, D.C., and Agu, V.O., 2017, Effect of ethanolic extract of *Chromolaena odorata* on the kidneys and intestines of healthy albino rats, *Integr. Med. Res.*, 6 (3), 292–299.

[5] Hu, J., Qi, Q., Zhu, Y., Wen, C., Olatunji, O.J., Jayeoye, T.J., and Eze, F.N., 2023, Unveiling the anticancer antimicrobial antioxidative properties

and UPLC-ESI-QTOF-MS/GC-MS metabolite profile of the lipophilic extract of siam weed (*Chromolaena odorata*), *Arabian J. Chem.*, 16 (7), 104834.

[6] Dew, L.A., Rozen-Rechels, D., le Roux, E., Cromsigt, J.P.G.M., and te Beest, M., 2017, Evaluating the efficacy of invasive plant control in response to ecological factors, *S. Afr. J. Bot.*, 109, 203–213.

[7] Alara, O.R., and Abdurahman, N.H., 2019, GC-MS and FTIR analyses of oils from *Hibiscus sabdariffa*, *Stima maydis* and *Chromolaena odorata* leaf obtained from Malaysia: Potential sources of fatty acids, *Chem. Data Collect.*, 20, 100200.

[8] Pel, P., Chae, H., Nhoek, P., Kim, Y.M., Khiev, P., Kim, G.J., Nam, J.W., Choi, H., Choi, Y.H., and Chin, Y.W., 2020, Stilbene dimer and flavonoids from the aerial parts of *Chromolaena odorata* with proprotein convertase subtilisin/kevin type 9 expression inhibitory activity, *Bioorg. Chem.*, 99, 103869.

[9] Onkaramurthy, M., Veerapur, V.P., Thippeswamy, B.S., Madhusudana R.T.N., Rayappa, H., and Badami, S., 2013, Anti-diabetic and anti-cataract effects of *Chromolaena odorata* Linn. in streptozotocin-induced diabetic rats, *J. Ethanopharmacol.*, 145 (1), 363–372.

[10] Eze, F.N., and Jayeoye, T.J., 2021, *Chromolaena odorata* (Siam weed): A natural reservoir of bioactive compounds with potent anti-fibrillogenic, antioxidative, and cytocompatible properties, *Biomed. Pharmacother.*, 141, 111811.

[11] Alara, O.R., Nour, A.H., and Abdul Mudalip, S.K., 2019, Screening of microwave-assisted-batch extraction parameters for recovering total phenolic and flavonoid contents from *Chromolaena odorata* leaves through two-level factorial design, *Indones J. Chem.*, 19 (2), 511–521.

[12] Omokhua, A.G., Ondua, M., van Staden, J., and McGaw, L.J., 2019, Synergistic activity of extracts of three South African alien invasive weeds combined with conventional antibiotics against selected opportunistic pathogens, *S. Afr. J. Bot.*, 124, 251–257.

[13] Omotuyi, O.I, Nash, O., Enejoh, O.A., Oribamise, E.I., and Adelakun, N.S., 2020, *Chromolaena odorata* flavonoids attenuate experimental nephropathy: Involvement of pro-inflammatory genes downregulation, *Toxicol. Rep.*, 7, 1421–1427.

[14] Omokhua-Uyi, A.G., Madikizela, B., Aro, A.O., Abdalla, M.A., Van Staden, J., and McGaw, L.J., 2023, Flavonoids of *Chromolaena odorata* (L.) R.M.King & H.Rob. as potential leads for treatment against tuberculosis, *S. Afr. J. Bot.*, 158, 158–165.

[15] Gade, S., Rajamanikyam, M., Vadlapudi, V., Nukala, K.M., Aluvala, R., Giddigari, C., Karanam, N.J., Barua, N.C., Pandey, R., Upadhyayula, V.S.V., Sripadi, P., Amanchy, R., and Upadhyayula, S.M., 2017, Acetylcholinesterase inhibitory activity of stigmasterol & hexacosanol is responsible for larvicidal and repellent properties, *Biochim. Biophys. Acta, Gen. Subj.*, 1861 (3), 541–550.

[16] Omokhua, A.G., McGaw, L.J., Finnie, J.F., and Van Staden, J., 2016, *Chromolaena odorata* (L.) R.M. King & H. Rob. (Asteraceae) in sub-Saharan Africa: A synthesis and review of its medicinal potential, *J. Ethnopharmacol.*, 183, 112–122.

[17] Nwachukwu, I., Aliga, C., Upabi, C.F., and Ukogo, I., 2016, *In-vitro* antibacterial effect of crude extract of *Chromolaena odorata* leaves on wound isolates, *IOSR J. Pharm. Biol. Sci.*, 11 (6), 49–52.

[18] Hridhya, K.V., and Kulandhaivel, M., 2017, Antimicrobial activity of *Chromolaena odorata* against selected pyogenic pathogens, *Int. J. Pharmacogn. Phytochem. Res.*, 9 (7), 1001–1007.

[19] Omokhua, A.G., McGaw, L.J., Chukwujekwu, J.C., Finnie, J.F., and Van Staden, J., 2017, A comparison of the antimicrobial activity and *in vitro* toxicity of a medicinally useful biotype of invasive *Chromolaena odorata* (Asteraceae) with a biotype not used in traditional medicine, *S. Afr. J. Bot.*, 108, 200–208.

[20] Vijayaraghavan, K., Rajkumar, J., and Seyed, A.M., 2018, Phytochemical screening, free radical scavenging and antimicrobial potential of *Chromolaena odorata* leaf extracts against pathogenic bacterium in wound infections– A multispectrum perspective, *Biocatal. Agric. Biotechnol.*, 15, 103–112.

[21] Yoplac, I., Vargas, L., Robert, P., and Hidalgo, A., 2021, Characterization and antimicrobial activity of microencapsulated citral with dextrin by spray drying, *Heliyon*, 7 (4), e06737.

[22] Lengyel, M., Kállai-Szabó, N., Antal, V., Laki, A.J., and Antal, I., 2019, Microparticles, microspheres, and microcapsules for advanced drug delivery, *Sci. Pharm*., 87 (3), 20.

[23] Bah, M.G., Bilal, H.M., and Wang, J., 2020, Fabrication and application of complex microcapsules: A review, *Soft Matter*, 16 (3), 570-590.

[24] Lombardo, S., and Villares, A., 2020, Engineered multilayer microcapsules based on polysaccharides nanomaterials, *Molecules*, 25 (19), 4420.

[25] Sharkawy, A., Fernandes, I.P., Barreiro, M.F., Rodrigues, A.E., and Shoeib, T., 2017, Aroma-loaded microcapsules with antibacterial activity for eco-friendly textile application: Synthesis, characterization, release, and green grafting, *Ind. Eng. Chem. Res*., 56 (19), 5516–5526.

[26] Xue, W., Zhang, M., Zhao, F., Wang, F., Goa, J., and Wang, L., 2019, Long-term durability antibacterial microcapsules with plant-derived Chinese nutgall and their applications in wound dressing, *e-Polym.*, 19 (1), 268–276.

[27] Sun, X., Cameron, R.G., and Bai, J., 2019, Microencapsulation and antimicrobial activity of carvacrol in a pectin-alginate matrix, *Food Hydrocolloids*, 92, 69–73.

[28] Cai, C., Ma, R., Duan, M., and Lu, D., 2019, Preparation and antimicrobial activity of thyme essential oil microcapsules prepared with gum arabic, *RSC Adv*., 9 (34), 19740–19747.

[29] Ambrosio, C.M.S., Alvim, I.D., Contreras Castillo, C.J., and Da Gloria, E.M., 2020, Microencapsulation enhances the *in vitro* antibacterial activity of a citrus essential oil, *J. Essent. Oil Bear. Plants*, 23 (5), 985–997.

[30] Julaeha, E., Puspita, S., Eddy, D.R., Wahydi, T., Nurzaman, M., Nugraha, J., Herlina, T., and Al Anshori, J., 2021, Microencapsulation of lime (*Citrus aurantifolia*) oil for antibacterial finishing of cotton fabric, *RSC Adv*., 11 (3), 1743–1749.

[31] Li, X., Gao, Y., Li, Y., Li, Y., Liu, H., Yang, Z., Wu, H., and Hu, Y., 2022, Formation of cinnamon essential oil/xanthan gum/chitosan composite microcapsules basing on Pickering emulsions, *Colloid Polym. Sci.*, 300 (10), 1187–1195.

[32] Demir, D., Goksen, G., Ceylan, S., Trif, M., and Rusu, A.V., 2023, Optimized peppermint essential oil microcapsules loaded into gelatin-based cryogels with enhanced antimicrobial activity, *Polymers*, 15 (13), 2782.

[33] Zhang, Q., Yang, A., Tan, W., and Yang, W., 2023, Development, physicochemical properties, and antibacterial activity of propolis microcapsules, *Foods*, 12 (17), 3191.

[34] Xu, Q., Song, L., Zhang, L., Hu, G., Chen, Q., Liu, E., Liu, Y., Zheng, Q., Xie, H., and Li, N., 2018, Synthesis of cellulose acetate propionate and cellulose acetate butyrate in a CO_2/DBU/DMSO system, *Cellulose*, 25 (1), 205–216.

[35] Carolin C, F., Kamalesh, T., Kumar, P.S., Hemavathy, R.V., and Rangasamy, G., 2023, A critical review on sustainable cellulose materials and its multifaceted applications, *Ind. Crops Prod*., 203, 117221.

[36] Pang, L., Gao, Z., Feng, H., Wang, S., and Wang, Q., 2019, Cellulose based materials for controlled release formulations of agrochemicals: A review of modifications and applications, *J. Controlled Release*, 316, 105–115.

[37] Obeidat, W.M., and Alizoubi, N.M., 2014, Controlled-release cellulose esters matrices for water-soluble diclofenac sodium: Compression and dissolution studies, *Pharmazie*, 69 (2), 96–103.

[38] Simões, M.G., Coimbra, P., Carreira, A.S., Figueiredo, M.M., Gil, M.H., and Simões, P.N., 2020, Eugenol-loaded microspheres incorporated into textile substrate, *Cellulose*, 27 (7), 4109–4121.

[39] Baldelli, A., Boraey, M.A., Nobes, D.S., and Vehring, R., 2015, Analysis of the particle formation process of structured microparticles, *Mol. Pharmaceutics*, 12 (8), 2562–2573.

[40] Varshosaz, J., Taymouri, S., Jafari, E., Jahanian-Najafabadi, A., and Taheri, A., 2018, Formation and characterization of cellulose acetate butyrate

nanoparticles loaded with nevirapine for HIV treatment, *J. Drug Delivery Sci. Technol.*, 48, 9–20.

[41] Amini Tapouk, F., Nabizadeh, R., Mirzaei, N., Hosseini Jazani, N., Yousefi, M., and Valizade Hasanloei, M.A., 2020, Comparative efficacy of hospital disfectants against nosocomial infection pathogens, *Antimicrob. Resist. Infect. Control*, 9 (1), 115.

[42] Topel, S.D., Balcioglu, S., Ateş, B., Asilturk, M., Topel, Ö., and Ericson, M.B., 2021, Cellulose acetate encapsulated upconversion nanoparticles - A novel theranostic platform, *Mater. Today Commun*., 26, 101829.

[43] Wang, W., Li, L., Jin, S., Wang, Y., Lan, G., and Chen, Y., 2020, Study on cellulose acetate butyrate/plasticizer systems by molecular dynamics simulation and experimental characterization, *Polymers*, 12 (6), 1272.

[44] Kwon, Y.R., Kim, H.C., Kim, J.S., So, J.H., Chang, Y.W., and Kim, D.H., 2022, Enhanced mechanical and thermal properties of chain-extended waterborne polyurethane coatings with cellulose acetate butyrate, *Polymers*, 14 (19), 4062.

[45] Oprea, M., and Voicu, S.I., 2020, Recent advances in composites based on cellulose derivatives for biomedical applications, *Carbohydr. Polym*., 247, 116683.

[46] Afiqah, S., Murtadza, A., Jai, J., Md Zaki, N.A., and Hamzah, F., 2021, Essential oils encapsulation performance evaluation: A review on encapsulation parameters, *MJCET*, 4 (2), 114–123.

[47] Wondrazek, H., Petzold-Welcke, K., Fardim, P., and Heinze, T., 2013, Nanoparticles from conventional cellulose esters: Evaluation of preparation methods, *Cellulose*, 20 (2), 751–760.

[48] Guastaferro, M., Cardea, S., Baldino, L., and Reverchon, E., 2021, Cellulose acetate nanocarrier production by supercritical assisted electrospray, *Chem. Eng. Trans*., 87, 391–396.

[49] Urbaniak, T., and Musiał, W., 2019, Influence of solvent evaporation technique parameters on diameter of submicron lamivudine-poly-ε-caprolactone conjugate particles, *Nanomaterials*, 9 (9), 1240.

[50] Peng, B., Almeqdadi, M., Laroche, F., Palantavida, S., Dokukin, M., Roper, J., Yilmaz, O.H., Feng, H., and Sokolov, I., 2019, Ultrabright fluorescent cellulose acetate nanoparticles for imaging tumors through systemic and topical applications, *Mater. Today*, 23, 16–25.

[51] Aprilia, N.A.S., Fauzi, F., Azmi, N., Najwan, N., and Amin, A., 2017, Performance of cellulose acetate membrane with different additives for palm oil mill effluent (POME) liquid waste treatment, *IOP Conf. Ser.: Mater. Sci. Eng.*, 334 (1), 12024–12030.

[52] Abdellah Ali, S.F., William, L.A., and Fadi, E.A., 2020, Cellulose acetate, cellulose acetate propionate and cellulose acetate butyrate membranes for water desalination applications, *Cellulose*, 27 (16), 9525–9543.

[53] Abu-Zurayk, R., Alnairat, N., Khalaf, A., Ibrahim, A.A., and Halaweh, G., 2023, Cellulose acetate membranes: Fouling types and antifouling strategies—A brief review, *Processes*, 11 (2), 489.

[54] Onda, A.J.A., Aquino, J.A., Mondala, P.A.B., and Bulatao, B.P.I., 2020, Evaluation of factors affecting the microencapsulation of mefenamic acid with cellulose acetate phthalate, *Pharm. Sci. Asia*, 47 (2), 130–141.

[55] Quintero, R.I., Galotto, M.J., Rodriguez, F., and Guarda, A., 2014, Preparation and characterization of cellulose acetate butyrate/oganoclay nanocomposites produced by extrusion, *Packag. Technol. Sci*., 27 (6), 495–507.

[56] Dairi, N., Ferfere-Harrar, H., Ramos, M., and Garrigós, M.C., 2019, Cellulose acetate/AgNPs-organoclay and/or thymol nano-biocomposite films combined antimicrobial/antioxidant properties for active food packaging use, *Int. J. Biol, Macromol*., 121, 508–523.

[57] Jeon, G.W., An, J.E., and Jeong, Y.G., 2012, High performance cellulose acetate propionate composites reinforced with exfoliated graphene, *Composites, Part B*, 43 (8), 3412–3418.

[58] Alghamdi, M.M., and El-Zahhar, A.A., 2021, Cellulose acetate butyrate graphene oxide nanocomposite membrane: Fabrication, characterization and performance, *Chem. Ind. Chem. Eng. Q.*, 27 (1), 35–44.

[59] Watanabe, T., Sakai, Y., Sugimori, N., Ikeda, T., Monzen, M., and Ono, T., 2022, Microfluidic production of monodisperse biopolymer microcapsules for latent heat storage, *ACS Mater. Au*, 2 (3), 250–259.

[60] Furtado, L.M., Hilamatu, K.C.P., Balaji, K., Ando, R.A., and Petri, D.F.S., 2020, Miscibility and sustained release of drug from cellulose butyrate/caffeine films, *J. Drug Delivery Sci. Technol.*, 55, 101472.

[61] Guimarães, T.L.F., da Silva, L.M.R., Lima, C.B., Magalhães, F.E.A., and de Figueiredo, E.A.T., 2020, Antimicrobial activity of microcapsules with aqueous extract of chambá (*Justicia pectoralis* Jacq), *Rev. Cienc. Agron.*, 51 (2), e20186471.

[62] Olawale, F., Olofinsan, K., and Iwaloye, O., 2022, Biological activities of *Chromolaena odorata*: A mechanistic review, *S. Afr. J. Bot.*, 144, 44–57.

[63] Vijayaraghavan, K., Rajkumar, J., Bukhari, S.N.A., Al-Sayed, B., and Seyed, M.A., 2017, *Chromolaena odorata*: A neglected weed with a wide spectrum of pharmacological activities (Review), *Mol. Med. Rep*., 15 (3), 1007–1016.

[64] Lobiuc, A., Pavăl, N.E., Mangalagiu, I.I., Gheorghită, R., Teliban, G.C., Amăriucăi-Mantu, D., and Stoleru, V., 2023, Future antimicrobials: Natural and functionalized phenolics, *Molecules*, 28 (3), 1114.

[65] Kauffmann, A.C., and Castro, V.S., 2023, Phenolic compounds in bacterial inactivation: A perspective from Brazil, *Antibiotics*, 12 (4), 645.

[66] Vital, P.G., and Rivera, W.L., 2009, Antimicrobial activity and cytotoxicity of *Chromolaena odorata* (L. f.) King and Robinson and *Uncaria perrottetii* (A. Rich) Merr. extracts, *J. Med. Plants Res.*, 3 (7), 511–518.

[67] Zayed, M., Othman, H., Ghazal, H., and Hassabo, A.G., 2021, *Psidium guajava* leave extract as reducing agent for synthesis of zinc oxide nanoparticles and its application to impart multifunctional properties for cellulosic fabrics, *Biointerface Res. Appl. Chem*., 11 (5), 13535–13556.

[68] Zhang, Y., Liu, X., Wang, Y., Jiang, P., and Quek, S., 2016, Antibacterial activity and mechanism of cinnamon essential oil against *Escherichia coli* and *Staphylococcus aureus*, *Food Control*, 59, 282–289.